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Biochimica et Biophysica Acta 1267 (1995) 145–151

BBA Biochimica et Biophysica Acta

Biochemical and pharmacological characterization of periodate-oxidized adenosine analogues at adenosine A_1 receptors

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Received 27 July 1994; revised 19 December 1994; accepted 8 March 1995

Abstract

Periodate oxidation of eight N^6-substituted adenosine derivatives was performed with the aim of oxidizing the vicinal 2′ and 3′ hydroxyl groups of the ribose moiety. A thermodynamical and pharmacological characterization of the products of this transformation allowed us to verify that oxidized adenosine analogues act as agonists at adenosine A_1 receptors. The dependence of their association constants on temperature indicates that their binding is entropy driven, a feature typical of adenosine A_1 receptor agonists; moreover all synthesized compounds were able to fully inhibit the forskolin induced c-AMP accumulation in rat isolated adipocytes. This is the first report suggesting that the presence of an intact ribose moiety is not necessary for agonistic activity at adenosine A_1 receptor. In fact periodate oxidation of the ribose moiety yields a dialdehyde and it is recognized that nucleoside dialdehydes are complex equilibrium mixtures of cyclic and acyclic hydrates and hemiacetals.

1. Introduction

Adenosine modulates a great variety of biological functions both in the nervous system and in pheripheral tissues [1]. Most of its effects appear to be mediated via two subtypes of specific membrane receptors, termed A_1 and A_2, which inhibit or stimulate, respectively, the adenylyl cyclase activity with consequent decrease or increase of the intracellular levels of the second messenger 3′-5′-cyclic adenosine monophosphate (c-AMP) [2,3]. More recently adenosine A_2 receptors have been divided into A_{2a} (high affinity) and A_{2b} (low affinity) subtypes and evidence for the existence of an A_3 receptor subtype has been raised [4].

Adenosine receptor agonists are closely related chemically to the endogenous ligand adenosine. Except for a limited number of modifications at the 5′ position, the intact ribose moiety seems to be an essential fragment for agonistic activity. Some adenine derivatives which lack the ribose moiety, such as 9-methyladenosine [5] and 9-phenyl-7-deazadenine [6] derivatives, are adenosine antagonists.

The structural requirements for the ribose moiety appear to be very strict: opening or enlargement of the pentose ring to a hexose ring are detrimental for affinity, as is inversion of the stereochemistry of the 5′-hydroxymethyl group [7]. The presence of a hydroxyl group on the 2′ position is essential for high affinity and high intrinsic activity. Removal of the hydroxyl groups at either the 3′ or the 5′ positions yields partial agonists that still have considerable affinity [7]. The 2′,3′-dideoxy analogue of CHA has been reported to act as a weak antagonist [8]. Recently, the 2′-deoxy and 3′-deoxy analogues of adenosine and N^6-substituted analogues have been proposed as partial agonists for the adenosine receptor [9] and the compound 6-cyclohexyl-2′-*O*-methyladenosine has been described as a potent and selective adenosine A_1 receptor agonist [10].

Binding thermodynamic analysis of adenosine agonists and xanthine antagonists at adenosine A_1 receptor revealed an entropy-driven binding of agonists and an enthalpy-driven binding of antagonists [11–13]. These data allowed to hypothesize a model of the adenosine A_1 receptor where the ribose moiety can replace and destroy a network of water molecules connecting two different domains of the

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SSDI 0167-4889(95)00045-3

A_1 receptor. According to this model the release of solvent molecules and the insertion of the ribose moiety would cause receptor conformation changes producing the final effect [13,14].

Ribose modification of 2′–3′ hydroxilic groups by sodium periodate has been instrumental in obtaining reactive nucleotides and nucleosides [15,16].

This paper deals with the pharmacological and biochemical characterization of compounds obtained after periodate oxidation of N^6-substituted adenosine derivatives which retain agonistic activity at the A_1 receptor. The agonistic behaviour of these modifidied ribose compounds, the first ever reported without an intact ribose ring, has been determined by measuring their capability to inhibit the forskolin-stimulated 3′-5′ cyclic adenosine monophosphate (c-AMP) accumulation in isolated epididymal rat adipocytes after stimulation by forskolin and will be discussed from a thermodynamical point of view.

2. Materials and methods

2.1. Materials

N^6-[^{3}H]Cyclohexyladenosine ([^{3}H]CHA: specific activity = 30.2 Ci/mmol) and Aquassure were obtained from NEN, Du pont de Nemours Italiana, (Milano, Italy). N^6-Cyclohexyladenosine (CHA), N^6-cyclopentyladenosine (CPA), N^6-*R*-phenylisopropyladenosine (R-PIA), N^6-*S*-phenylisopropyladenosine (S-PIA), N^6-phenyladenosine (PhADO), N^6-benzyladenosine (BzADO), N^6-phenylethyladenosine (PhEtADO), N^6-methyladenosine (MeADO) and 1,3-dipropyl-8-cyclopentylxantine (DPCPX) were purchased from Sigma, St. Louis, MO. Male Wistar rats were acquired from Nossan Laboratories (Varese, Italy). Bovine serum albumin (fraction V), forskolin, adenosine deaminase (type VI), aminophylline, 3′-5′-cyclic monophosphate (c-AMP) and collagenase (type II) were obtained from Sigma (St. Louis, MO, USA). Ro 20-1724 (4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone) was a kind gift of Dr. E. Kyburz, Hoffmann-La Roche (Basel, Switzerland). [^{3}H]c-AMP (specific activity = 24 Ci/mmol) was obtained from Amersham (Milano, Italy). Ready Gel was obtained from Beckman (Irvine, CA, USA).

Unless otherwise stated, other materials were from standard sources.

2.2. Synthesis of oxidized adenosine analogues

Synthetic procedure was performed to obtain periodate-oxidized derivatives of CHA (CHAox), CPA (CPAox), R-PIA (R-PIAox), S-PIA (S-PIAox), PhADO (PhADOox), BzADO (BzADOox), PhEtADO (PhEtADOox) and MeADO (MeADOox). Oxidized adenosine analogues preparation was carried out essentially as described by Gilham [17]: 1 mM N^6-substituted adenosine analogues and 1 mM metaperiodate cold solutions in water, pH 6.0, were mixed together and the reaction was allowed to proceed for 1 h at 0° C in the dark. The reaction was monitored by thin-layer chromatography on silica with methanol/chloroform (1:9) as developing solvent. Iodate was removed by filtration through Sep-Pak Cartridge, the oxidized product was eluted with acetonitrile and the solvent was evaporated to dryness.

To confirm the purity of the oxidized products, N^6-substituted adenosine nucleotides and the corresponding ribosil oxidized derivatives were analyzed at room temperature by HPLC. A Beckman high pressure liquid chromatograph (Sistem Gold programmable Solvent Module 126) and a Beckman ODS (4.6 mm × 25 cm) column were used. During the first 4 min, (flow rate 1 ml/min) water was used as solvent, than a linear water-methanol gradient at a constant flow rate was used for the evaluation of the retention times of nucleosides and oxidized analogues. All compounds were injected as their hydrochloride salts dissolved in aqueous solution at concentrations of approx. 0.4 mg/ml. 100 μl aliquots were applied to column chromatography.

Spectral properties of adenosine dialdehyde were assigned by IR and NMR measurements. IR spectra were taken on paraffin oil mulls on a Perkin-Elmer Model 1310 instrument. [^{1}H]NMR spectra were detected with a Varian CFT-20 instrument operating at 80 MHz in a 2% D_2O solution, using Me_4Si as the internal standard.

2.3. Membrane preparation

Male Wistar rats (150–200 g) were decapitated and the whole brain (minus brainstem, striatum and cerebellum) was dissected on ice. The tissue was disrupted in a Polytron (setting 5) in 20 vols of 50 mM Tris-HCl, pH 7.4. The homogenate was centrifuged at 48 000 × *g* for 10 min and the pellet was resuspended in the same buffer, centrifuged and resuspended in Tris-HCl containing 2 IU/ml adenosine deaminase (type VI). After 30 min incubation at 37° C the membranes were centrifuged and pellets were stored at −70° C. Prior to freezing, an aliquot of homogenate was removed for protein assay [18].

2.4. Preparation of rat adipocytes

Isolated rat fat cells were prepared essentially according to the method of Rodbell [19]. Wistar male rats (250–300 g) were killed by decapitation. The epididymal fat pads were quickly removed, washed in physiological solution and immediately placed in a beaker with a Krebs-Ringer bicarbonate buffer containing NaCl 125 mM, KCl 5 mM, $CaCl_2$ 1 mM, KH_2PO_4 1 mM, $MgSO_4 \cdot 7H_2O$ 1.2 mM, glucose 0.1%, 1% bovine serum albumin (fraction V), gassed with 5% CO_2 and 95% O_2. The pads were cut in small pieces and transferred, with buffer, into a plastic beaker containing 1 mg/ml of collagenase (type II). The

mixture was incubated for 45 min at 37° C in a shaking bath. The contents were filtered twice through a nylon sieve to remove undigested tissue. Adipocytes were then washed two or three times with buffer and then centrifuged at $100 \times g$ for 15 s. Finally diluted aliquots of fat cells were counted under an optical microscope.

2.5. Receptor binding assays

Binding assays were performed at 0, 10, 20 and 25° C essentially according to Bruns et al. [20]. Displacement experiments were performed in 1 ml of buffer containing 1 nM [^{3}H]CHA and membranes from 15 mg (wet weight) of tissue. To determine IC_{50} values (where IC_{50} is the inhibitor concentration displacing 50% of the labelled ligand), fresh solutions of the test compounds were added in triplicate to the binding assay samples at a minimum of six different concentrations. Saturation binding experiments were carried out using 8 to 12 concentrations of [^{3}H]CHA ranging from 0.1 to 10 nM. Separation of bound from free radioligand was performed by rapid filtration through Whatman GF/B filters which were washed three times with ice-cold buffer. Filter bound radioactivity was measured by scintillation spectrometry after addition of 4 ml of Aquassure. Non-specific binding was defined as the binding in presence of 10 μM (*R*)-N^6-phenylisopropyl adenosine (R-PIA); this is always < 10% of the total binding. The incubation time ranged from 2.5 h at 0° C to 1.75 at 25° C according to the results of previous time course experiments. K_i values were calculated from the Cheng and Prusoff equation [21]. All binding data were analysed using the non-linear regression curve fitting computer program LIGAND [22].

2.6. Calculations

For a generic binding equilibrium $L + R = LR$ (L = Ligand, R = Receptor), the affinity constant is calculated as $K_a = [LR]/([L][R]) = [LR]/([L_{max} - LR][B_{max} - LR]) = 1/K_d$, where $[L_{max}]$ = total concentration of the ligand added, $[B_{max}]$ = total concentration of the binding sites and K_d = dissociation constant. As $[LR]/[L_{max} - LR] = [Bound]/[Free] = [B_{max}]K_a - K_a[Bound]$, the K_a and B_{max} values can be obtained from the slope and intercept of the Scatchard plot [Bound]/[Free] versus [Bound]. The standard free energy was calculated as $\Delta G^\circ = -RT \ln K_a$ at 298.15 K, the standard enthalpy, ΔH°, from the van t'Hoff plot $\ln K_a$ versus $(1/T)$ (whose slope is $-\Delta H^\circ/R$) and the standard entropy as $\Delta S^\circ = (\Delta H^\circ - \Delta G^\circ)/T$ with $T = 298.15$ K and $R = 8.314$ J/K per mol.

2.7. Determination of cyclic AMP levels

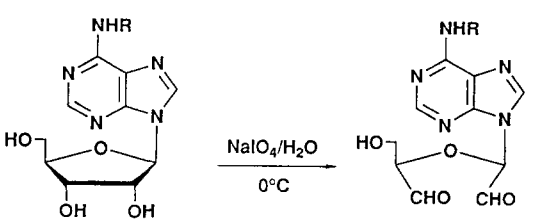
10^5 fat cells were suspended in 400 μl of Krebs-Ringer buffer, pH 7.4, containing 1 IU/ml of adenosine deaminase (type VI), 0.5 mM Ro 20-1724 as phosphodiesterase inhibitor and incubated for 10 min at 37° C in a shaking bath. Then adenosine A_1 receptor agonists and forskolin at 1 μM final concentration were added and, after a 5 min time of incubation, the reaction was stopped by adding ice-cold 6% (w/v, final concentration) of trichloroacetic acid. Trichloroacetic acid suspensions were centrifuged at $2000 \times g$ for 10 min at 4° C and the acidic supernatant was extracted four times with water-saturated ethyl ether. The final aqueous solution was tested for c-AMP by a competitive protein binding assay carried out essentially according to Brown et al. [23] and Nordstet and Fredholm [24]. The sample (100 μl) or 50 μl c-AMP standard (0–10 pmol) was added at each test tube containing Trizma base 100 μM, aminophylline 8 mM, 2-mercaptoethanol 6 mM at pH 7.4.

[^{3}H]c-AMP (1 pmol, approx. 25 000 cpm) was added to each tube at a total assay volume of 500 μl. Binding protein, previously prepared from beef adrenals essentially according to Brown et al. [23], was added to the samples. They were incubated at 4° C for 150 min and, after the addition of albumin saturated charcoal, centrifuged at $2000 \times g$ for 10 min. 200 μl aliquots of clear supernatant were mixed with 4 ml of Ready Gel and counted in a Beckman liquid scintillation spectrometer (Beckmann LS 1800 Irvine, CA, USA) at a counting efficiency of about 55%. Results were expressed as pmol of c-AMP per 10^5 adipocytes.

IC_{50} values in the c-AMP assays were obtained for fresh solutions of the compound under examination from the concentration-inhibition curves by linear regression after logit-log transformation.

3. Results

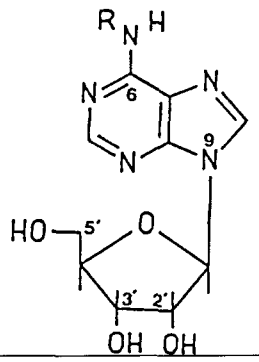
All selected N^6-substituted adenosine dialdehydes, which were prepared as potential adenosine A_1 receptor competitive ligands, were obtained by oxidation of the corresponding ribonucleosides using 1 equivalent of sodium metaperiodate in H_2O (Scheme 1).



Scheme 1.

These ribonucleoside dialdehydes were quite unstable under the acidic reaction condition (pH 6), therefore, after reaction, iodate was removed by filtration through Sep-Pak Cartridge, and the oxidized product was eluted with acetonitrile and the solvent was evaporated to dryness. Usual

Table 1
Inhibitory binding constants at 25° C, K_i (nM), for the displacement of [^{3}H]CHA from rat brain adenosine A_1 receptors by a selection of adenosine agonists and periodate-oxidized analogues



Compound	R	K_i	Periodate oxidized compound	K_i
CHA	Cyclohexyl	1.0(±0.03)	CHAox	9.0(±0.4)
CPA	Cyclopenthyl	0.03(±0.001)	CPAox	1.03(±0.06)
R-PIA	Phenylisoproyl	0.60(±0.03)	R-PIAox	6.2(±0.3)
S-PIA	Phenylisopropyl	11.7(±0.9)	S-PIAox	300(±18)
PhADO	Phenyl	4.1(±0.3)	PhADOox	38(±3)
PhEtADO	Phenylethyl	11.5(±0.9)	PhEtADOox	100(±9)
BzADO	Benzyl	57.9(±4)	BzADOox	930(±47)
MeADO	Methyl	126(±11)	MeADOox	1,540(±76)

Values are means (±S.E.) of four separate experiments.

crystallization and characterization by chemical analysis were prohibited by the low amounts of the compounds. In an effort to ensure the absence of the starting material in miscellaneous with oxidized product, we performed a chromatographic characterization of the N^6-substituted adenosine nucleosides and the corresponding oxidized analogues. All selected compounds, before and after oxidation demonstrated a single spot at TLC analysis and a single peak at HPLC analysis, carried out as described in Section 2. Retention Factor values, (TLC) and Retention Time values, (HPLC) were different for tested adenosine analogues before and after oxidation (data not shown). Chromatographic properties of all oxidized compounds remained unchanged when the dried products were stored at 5° C at least for 3 months.

In an effort to elucidate the chemical structure of N^6-substituted adenosine dialdehydes in solution, we investigated the spectral properties (IR and NMR) of adenosine dialdehyde, which was obtained by periodate oxidation of the corresponding ribonucleoside, as previously reported [25]. The oxidized product did not exhibit the expected carbonyl stretching vibrations in the IR region (1700 cm^{-1}), nor did it exhibit free aldehydic protons in the NMR (σ 8.5–10.0). Instead, we observed hydroxyl stretching vibrations (3300 cm^{-1}) in the IR, and the NMR showed the C2′ and C3′ protons upfield in the range of δ

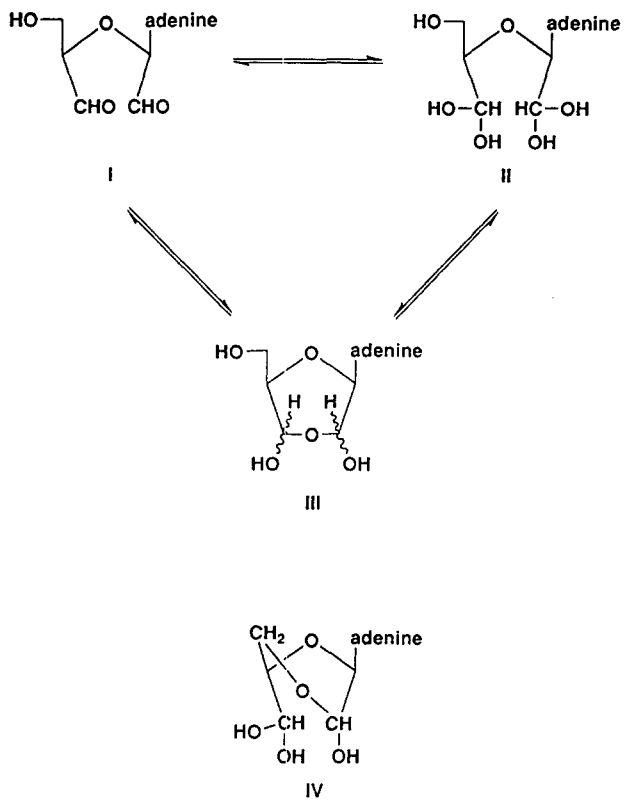
Table 2
Equilibrium binding parameters at four different temperatures for the displacement of [^{3}H]CHA from rat brain adenosine A_1 receptors by eight N^6-substituted 2′,3′-oxo-adenosine derivatives

Ligand	T(K)	273	283	293	298
	T(° C)	0	10	20	25
[^{3}H]CHA	K_d	4.4(±0.2)	1.6(±0.1)	1.3(±0.05)	1.04(±0.04)
	B_{max}	198(±16)	208(±15)	202(±14)	210(±12)
CHAox	K_i	48(±3)	12.6(±0.5)	9.8(±0.5)	9.0(±0.4)
CPAox	K_i	5.4(±0.3)	4.1(±0.2)	2.04(±0.1)	1.03(±0.06)
R-PIAox	K_i	17.1(±0.9)	8.8(±0.4)	5.5(±0.3)	6.2(±0.3)
S-PIAox	K_i	1460(±70)	503(±27)	382(±21)	300(±18)
PhADOox	K_i	179(±8)	80(±5)	60(±4)	38(±3)
BzADOox	K_i	2640(±154)	1380(±75)	1030(±58)	930(±47)
PhEtADOox	K_i	447(±21)	308(±16)	229(±11)	100(±9)
MeADOox	K_i	4800(±300)	1800(±94)	1530(±80)	1540(±76)

Dissociation, K_d, and inhibition, K_i, constants in nM and B_{max} in fmol/mg of protein. Values are means ± S.E. of four experiments.

5.2–6.0. These spectral data suggest that the dialdehydes probably exist in various hydrated forms rather than as free aldehydes.

Scheme 2 reports the possible structures of adenosine dialdehyde and related compounds which exist at equilibrium in aqueous solution.



Scheme 2.

Possible structures include the hydrated form II and the various possible diastereoisomeric forms of hydrated species III. The hydrated structure IV is also possible because of the additional 5′-hydroxyl group. They exist in deuterium oxide as an almost 1:1 mixture of the acyclic hydratated dialdehyde (II) and the hemiacetal (III, IV) form.

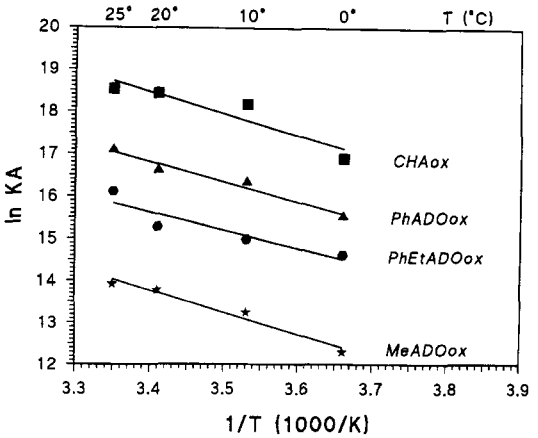


Fig. 1. Representative van't Hoff plots showing the effect of temperature on the equilibrium association constants, K_a, for a selection of four oxidized adenosine derivatives. All plots are essentially linear ($r \geq 0.96$) in the temperature range from 0° C to 25° C.

All selected compounds were evaluated for A_1 adenosine receptor affinity as measured by the ability to displace 1 nM [^{3}H]CHA from rat brain adenosine A_1 receptors at 25° C. The results are shown in Table 1. For each of the oxidized adenosine derivatives tested, at the chosen temperatures, K_i values were systematically greater than those of the equivalent N^6-monosubstituted analogues which have been previously reported in [13].

Table 2 reports the values of inhibitory binding constant, K_i, obtained at the four chosen temperatures for the selected eight ligands; K_d and B_{max} values, derived from saturation experiments carried out with [^{3}H]CHA in the range 0.1–10 nM, are also shown. Since K_i values can be considered equivalent to the dissociation equilibrium constants, K_d, the K_d value measured for [^{3}H]CHA can be considered to be homogenous with the other tabulated data. B_{max} values obtained from saturation experiments appear to be largely independent of temperature, at variance with inhibitory binding constants which change with

Table 3
Thermodynamic parameters for displacement of [^{3}H]CHA, by N^6-substituted 2′,3′-oxo-adenosine derivatives, from rat brain adenosine A_1 receptors and IC_{50} values in the c-AMP assays obtained from the concentration-response curves by linear regression analysis after logit-log transformation

Compound	$\Delta G°$ (kJ/mol)	$\Delta H°$ (kJ/mol)	$\Delta S°$ (J/mol per K)	IC_{50} (nM) c-AMP assay
CHAox	−46.3 ± 0.3	42 ± 10	297 ± 38	8.1 ± 0.7
CPAox	−51.7 ± 0.3	44 ± 9	320 ± 32	0.23 ± 0.01
R-PIAox	−47.6 ± 0.3	30 ± 6	259 ± 23	2.2 ± 0.1
S-PIAox	−37.2 ± 0.3	40 ± 8	260 ± 29	11 ± 1
PhADOox	−42.0 ± 0.2	39 ± 5	271 ± 18	1.5 ± 0.2
BzADOox	−34.5 ± 0.2	28 ± 4	210 ± 13	2000 ± 150
PhEtADOox	−39.9 ± 0.3	35 ± 10	249 ± 36	510 ± 48
MeADOox	−33.5 ± 0.3	29 ± 10	210 ± 34	3000 ± 280

Values are means ± S.E. of four separate experiments.

changing temperature. The temperature dependence of affinity constants, $K_a = 1/K_i$, is exemplified by the van't Hoff plots ln K_a versus $1/T$ of Fig. 1, reporting typical results for four compounds (CHAox, PhADOox, PhEtADOox, MeADOox). Van't Hoff plots appear to be essentially linear in the range 0–25° C for all selected compounds. The linearity of the van't Hoff plots indicates that ΔC_p° is nearly zero seemingly a general property of most classical membrane receptor so far studied [26]. The slopes of the van't Hoff plots are consistently positive for the selected compounds whose affinities are improved by a temperature increase (Fig. 1). Final thermodynamic parameters calculated for the binding equilibria of the different compounds investigated are reported in Table 3. ΔG° values range from -51.7 to -33.5 kJ mol^{-1}, while equilibrium standard values of enthalpy, ΔH°, and of entropy, ΔS° show that the binding of the selected compounds is totally entropy-driven ($28 \leq \Delta H^\circ \leq 44$ kJ mol^{-1}; $210 \leq \Delta S^\circ \leq 278$ J mol^{-1} K^{-1}). Referring to the thermodynamic discrimination between adenosine agonists and xanthine antagonists [11–13], the entropy-driven binding of the oxidized derivatives indicates that their mechanism of interaction with adenosine A_1 receptor is similar to that of adenosine analogues, thus suggesting an agonist behaviour. These indications are confirmed by the capability of all oxidized adenosine derivatives to fully inhibit the forskolin induced c-AMP accumulation in rat isolated adipocytes. The inhibition was fully blocked by the specific adenosine A_1 receptor antagonist, DPCPX, which proves an adenosine A_1 receptor mediated effect. The IC_{50} values in the c-AMP assay, reported in Table 3, are obtained from the concentration-response curves, reported in Fig. 2, by linear regression analysis after logit-log transformation and range from 0.23 nM to 3000 nM.

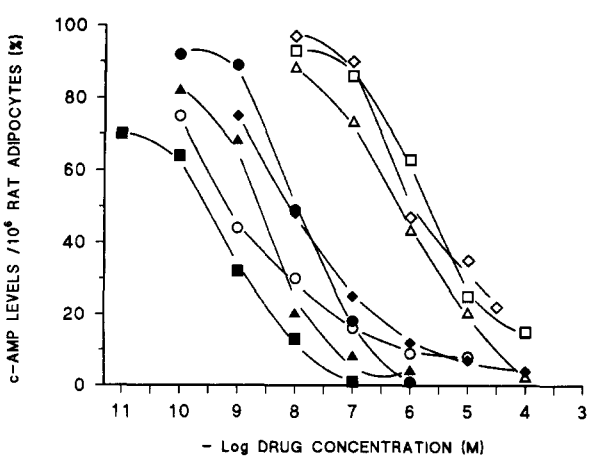


Fig. 2. Inhibition of forskolin-stimulated c-AMP levels in rat isolated adipocytes by oxidized adenosine derivatives (●, CHAox; ■, CPAox; ▲, R-PIAox; ◆, S-PIAox; ○, PhADOox; □, BzADOox; △, PhEtADOox; ◇, MeADOox). This is a single representative experiment carried out in duplicate.

4. Discussion

The data accumulated in the literature on molecular modifications of adenosine structure indicate the physiological activity mediated by specific receptors to be limited largely to analogues with intact purine ring and β-ribofuranosyl moieties. The adenine moiety is reported as being more amenable to substitutions than the ribose moiety. A number of useful modifications may be performed at C^2 and N^6 amino group [27–29]. In particular N^6-substituted analogues of adenosine have generally proven to be A_1-receptor selective with N^6-cyclohexyladenosine (CHA) being 400–800 fold selective [30].

Furthermore, according to reports in the literature, few alterations of the ribose moiety are tolerated at the adenosine receptor binding site [5]. The 5′ position can be modified to some extent to yield compounds that retain high affinity. The best known examples are 5′-(*N*-ethyl)carboxamidoadenosine (NECA), 2-hexynyl-5′-(*N*-ethyl) carboxamidoadenosine (HE-NECA) and 2-[*p*-(carboxyethyl)-phenethylamino]-5′-(*N*-ethyl) carboxamidoadenosine (CGS 21680), all containing a 5′-*N*-ethylcarboxamido group. The modification at N^9 include deoxy sugars, 5′-substituted-5′-deoxyriboses, non-ribose sugars, sugar ring homologues, and acyclic sugar analogues having poor affinity for adenosine receptors. Only minor modifications at 3′ position maintain potent binding [7].

However, the present paper demonstrates that the periodate oxidation of N^6-substituted adenosine derivatives allows maintenance of a specificity and a typical agonistic behaviour similar to that of the parent compounds which has been characterized by an entropy driven binding [11–13] and by the capability to inhibit the forskolin induced c-AMP accumulation in rat isolated adipocytes. The entropy-driven binding associated to the agonistic behaviour can be explained by hypothesizing the substitution from the binding site of a network of water molecules with a bulky hydrophilic fragment: the ribose moiety, in the case of adenosine derivatives [13,14], and by a fragment different from ribose after periodate oxidation. This is the first report about the existence of adenosine A_1 agonists lacking an intact ribose linked to the nitrogen at position 9 of the adenosine pyrimidine nucleus. Some considerations are in order regarding the characterization of the fragment derived after the periodate oxidation of the ribose moiety. In fact oxidation of single glycosides such as methyl α-D-glucopyranoside with sodium metaperiodate yields a dialdehyde [31,32]. However, it is well documented that after solubilization nucleoside dialdehydes are capable to exist in a variety of modifications, depending on such conditions as the type of solvent [25]. In water or buffer they may exist as complex equilibrium mixtures of cyclic and acyclic hydrates and hemiacetals [25]. However, since crude product did not display absorption in the infrared region of the spectrum characteristic of aldehyde groups, it seems probable that the products exist as various acetals.

These results are confirmed by the generally recognized fact that nucleoside dialdehydes are really complex equilibrium mixtures of cyclic and acyclic hydrates and hemiacetals, as previously reported [33–35].

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